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Effectiveness of Phenol Compound Isolated from *Menthe spicata* Leaves and Nano – Zinc Oxide on Antimicrobial Activity

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Abstract: The aim from this the study effect Phenol compound isolated from *Menthe spicata* leaves and Nano – Zinc Oxide on Antimicrobial activity antifungal (*Candida albicans*) and antibacterial (*staphylococcuse aureas* and *Escherichia coli*) , The *Menth spicata* plant collection from the city of Karbala in 2/9/ 2022 after cleaning and removing soil. As the seed were washed three times with tap water , extraction by using soxhelet apparatus by using methanol (70%) , through 24 hours of and evaporation by rotary evaporation apparatus 40C° for drying, and isolation phenol from *Menthe spicata* leaves , *E. coli* and *Sta. aureus* and *C.albicans* were obtained from libraries university of karbala , the antimicrobial test against (*S.aureas* , *E.coli* and *C.albicans*) was detected by disc diffusion methods was carried out using phenol extract at concentrations of 0.5, 1, 2, and 13 mg/ml , while Znonanoparticles was performed 0.2, 0.5, 0.8, and 1 mg/ml concentration . indicate the results the inhibition zone of phenol was 17.5 against *C.albicans* , 13.76 mm against *E .coli*, 12.87 mm against *S. aureus* in concentration (0.5) mg/ml. while concentration 1 mg/ml in *C.albicans* 18.34 , *S. aureus* 13.98mm , *E.coli* 14.98mm , while concentration 2mg/ml in *C.albicans* 19.21mm , *S. aureus* 14.98mm , *E.coli* 16.87 mm , concentration 3 mg/ml in *C.albicans* 21.65mm *S. aureus* 16.87 mm , *E.coli* (17.98) mm. While Znonanoparticles' *C.albicans* inhibition zone was 18.98, , 19.76 mm against *E .coli*, 20.76 mm against *S. aureus* in concentration 0.2 mg/ml, while concentration 0.5 mg/ml in *C.albicans* 19.32 , *S. aureus* 21.87mm , *E.coli* 20.54mm , while concentration 0.8mg/ml in *C.albicans* 21.87mm , *S. aureus* 22.87 , *E.coli* 16.87 mm , concentration 1 mg/ml in *C.albicans* 22.98mm , *S. aureus* 24.8 mm and *E.coli* 23.98 mm. The study's finding is that *C.albicans* was inhibited by phenol compounds at various doses., while the Znonanoparticles with various doses caused the antibacterial activity to be inhibited (*S.aureas* , *E.coli* and *C.albicans*).

Keywords*: *Phenol, Menthe spicata, Nano – Zinc Oxide..

1.Introduction

Candida albicans was a generally benign microflora in healthy individuals, but in immunocompromised pati -ents, it transforms into an opportunistic pathogen that can cause a potentially fatal widespread infection [1]. A frequent opportunistic disease of the skin and oral cavity caused by a type of Candida is called candidiasis. C. albi -cans is the most prevalent and widely dispersed, along with C. guilliermondii, C. dubliniesis, C. tropic -alis, and C. krusie [2]. Due to the presence of the causal agent, primarily C. albicans, as a commensal fungal in the oral cavity, vagina, and digestive tract, candidiasis is a comm -on recurrent infection [3]. Due to the presence of the causal agent, primarily C. albicans, as a commensal fungal in the oral cavity, vagina, and digestive tract, candidiasis is a common recurrent infection [4]. There fore, the eradication of both antibiotic-susceptible andresistant strains of Candida albicans may require a non-antibiotic treatment that is very effective and safe. There are numerous papers that demonstrate natural products' antifungal effectiveness [5]. C. albicans cells have protective cell walls that facilitate interaction with

the host for adherence and control of the host's antifungal immune response [6]. Due to their toxicity on the liver and pancreases when used at high concen - trations, various plant medicines have been used as natural anti-fungal formulations in place of synthetic anti-fungi that are used as skin ointments [7].

Additionally, these plants are regarded as a storehouse of numerous potent compounds, including volatile and essential oils, ketones, and tannins that are formed as secondary metabolites [8]. The menthe plant is a fragrant perennial that is common in tropical and subtropical climates and a source of terpene-rich esse ntial oils [9]. Finding novel antibacterial medicines has become necessary due to the emergence of bacterial resistance to currently existing antibiotics. The majority of post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis, and food poiso - ning are caused by gram positive bacteria like Staphyl ococcus aureus. Throughout the history of the human population, infections have been the main reason why people get sick. It was believed that once antibiotics were available, this issue would be solved. However, bacteria have managed to mutate so that they are no longer susceptible to antibiotics [10]. The use of novel compounds that are not based on synthetic antimicrobial agents already in use is the only method to prevent antibiotic resistance [11].

M. spicata belong to Lamiaceae family and are important sources of biologically active compounds [12]. The dried leaves are usually used for herbal tea or as a medication, whereas the fresh leaves are typically consumed as a raw vegetable or flavoring herb. Several biological effects of mint herbs have been linked to them, including antioxidant, anti-inflammatory, anti-cancer, and antibacterial properties [13].

Natural phenolic compounds are widely distributed throughout the plant world. They can build up significantly in some organs or tissues of the plant and can be found in leaves, fruits, bark, and wood [14].

They are divided into three categories: phenolic acids, flavonoid polyphenolics (catechins, flavonones, and flavones), and non-flavonoid polyphenolics [15]. Zinc Oxide Nanoparticles is one of the most essential microelemental indispensable vital functions. It comes from water and food to the organism and, mainly absorbed in small intestine and transport to the blood plasma [16].

Many industries and medications use zinc oxide nanoparticles (ZnO-NPs), which have potential uses like antifungal, antibacterial, anti-diabetic, anti-inflammat - ory, wound healing, and antioxidant. Green approaches using plants, fungi, bacteria, and algae have also been employed [17].

2. Methodology

- **Plant Collection**

M. spicata plant was taken from the city of Karbala in 2/9/ 2022 after cleaning and removing soil. As the seed were washed three times with tap water and once with distilled water. After that, dry out in the shade. Each seeds dry component underwent electrical grinding. Up to the time of usage, the powdered components were stored in plastic tubes in the refrigerator at 40C [18].

- ***Alcoholic Extracts***

Alcoholic extract was made by using 100g of powdered seeds, 100ml of methanol alcohol solvent (70%) in a 500 ml flask for extraction by using soxhelet apparatus , thr - ough 24 hours of and evaporation by rotary evaporation 40C° for drying, and storage at 4C° until use [19] . The percentage is estimated using the law shown in equation No (1) [20].

$$\text{Yield (wt\%)} = \frac{\text{Weight of Oil Produced}}{\text{Weight of Seed used}} \times 100\%$$

Separation Phenolic Compounds

- Take methanolic extract acidify with (2M)HCL (PH <3).
- Put extract in separation funnel and washed by chloroform (CHCL3).
- Mixing .
- Forming two layer , Take lower layer .
- Repeat this steps two or three time.
- Phenols collection was dried in oven (30 – 20) °C .
- Until used, phenol is kept in a refrigerator at 4°C [21].

Identification of Phenolic Compounds

- Preliminary qualitative test: The identification of phenol compounds were implemented using several tests such as : Ferric Chloride reagent lead acetate reagent
- Reagents for ferric chloride Three to four drops of a ferric chloride solution were added to the extracts. The presence of phenols is indicated by the formation of a bluish-black color [22].
- acetate of lead Using distilled water, the extract (50 mg) was dissolved in 5 ml. 3ml of 10% lead acetate was then added to it. The presence of phenol compounds was indicated by an obtrusive white precipitate [23].

Secondary Metabolism Screen Study

- ***Saponins***

The studied samples (2.5 mL) were added to sterile distilled water (10 mL) in a test tube to identify saponins using the foam index method.It was then covered and aggressively shook for around 40 seconds. They were permitted to remain standing for perhaps 30 minutes after that.The presence of saponins is shown by the honey comb foam.

- ***Phenols***

After applying 0.5 ml of 1% lead acetate solution to 5 mg of oil extract during the lead acetate test, precipitate was produced.

- ***Glycosides***

Aqueous NaOH solution was added after 0.5 mg of parsley oils extract was dissolved in 1 ml of water to produce the color yellow.

- ***Tannins***

Distilled water (D.W) was added to 5 ml of oil extract, which was then heated at 80–100 °C for 10 minutes in a water bath apparatus. After filtering, the mixture was colored dark green by adding 5 drops of 1% ferric chloride.

- ***Alkaloids***

The collected filtrate was combined with Wagner reagent, a potassium iodide and iodine solution. production of a reddish-brown precipitate.

- ***Flavonoids***

1.5ml of 50% methanol was added to 4mL of extracts. 5–6 drops of concentrated HCl were added after mixing, and the resulting mixture was heated with magnesium metal till red color was produced. There are flavonoids when a color is red [23].

Biological Effect:

- ***Specimens Collection of Bacteria***

Escherichia coli and *Staphylococcus aureus* were obtained from libraries university of karbala. The bacteria were turned on, grown in nutrient agar, and then kept at 4 °C on nutritional agar slants [24].

- ***Staph Aureus and Echericia Coli***

Were kept after being identified by transferring a single, isolated, pure colony to nutrient agar slant in a screw-capped tube, incubating it overnight at 37°C, and then briefly storing it in the refrigerator at 4°C [25].

- ***Isolation and Identification of C.albicans***

Isolate of *C.albicans* was obtained from Postgraduate fungal laboratory, Karbala University. The samples were activated and cultured on sabouraud dextrose agar that contains chloram phenicol to prevent bacteria contam - ination and incubated 37°C . *C. albicanes* was identified on the basis of colony morphological characteristics on Candida-chrom agar media and germ tube production in fresh serum .

- ***Zno Nanoparticals***

Were purchased from the Nanomaterials , Pratical size (33-40nm) (0.2, 0.5, 0.8, and 1 mg/ml) at various dosages.

Statistical Analysis

Mean was used to express the data.One-way analysis of variances was used to assess the statistical significance of differences between the control and other groups (ANOVA).The SPSS for Windows version was used for statistical analysis, and P values of 0.05 or less were considered significant (SPSS, Inc., Chicago, Illinois).

Results

Percentage Yield of Alcohol Extract and Phenol Extracted:

The results of table (1) showed that Percentage yield of Alcohol extract for *M. spicata* was at its proportion (4%), while Percentage yield of Al phenol for *M. spicata* was at its proportion (1.8%).

Table 1. Percentage yield of Alcohol extract and phenol extracted.

Extracted	Alcohol extract	Phenol
M. spicata	4/100*100=4%	1.8/100*100=1.8%

Phytochemical Reagents Study of Alcohol Extracted for M.spicata :

The result of phytochemical screening of methanol alcohol extract of *M. spicata* in table (2) showing methanol alcohol extract that high positive reaction with used reagent , Alkaloid , Phenol, , Glycoside, Flavonoid , Saponin and tannin.

Table 2. Phytochemical reagents of *M.spicata.*

Phytochemical reagents	Alcohol extract
Saponin	+
Phenol	+
Glycoside	+
Flavonoid	+
Alkaloid	+
Tannin	+

Table 3. Shows the type of extracts that interact and their concentrations in the zone of inhibition for phenol from *M. spicata* on *C.albicans* fungus development.

Concentrations mg/ml	Zone inhibition			Control
	C.albicans	*E.coli*	*S. aureas*	
0.5	17.5	13.76	12.87	0
1	18.34	14.98	13.98	0
2	19.21	16.87	14.98	0
3	21.65	17.98	16.87	0

LSD (0.01) =	1.65	1.87	1.91

Table 4. Shows the type of extracts that interact and their concentrations in the zone of inhibition for Zno nanoparticles on *C.albicans* fungus development.

Concentrations mg/ml	Zone inhibition			Control
	C.albicans	*E.coli*	*S. aureas*	
0.2	18.98	19.76	20.76	0
0.5	19.32	20.54	21.87	0
0.8	21.87	21.87	22.87	0
1	22.98	23.98	24.8	0
LSD (0.01) =	1.64	1.43	1.98	

The disc diffusion method was employed in this investigation to ascertain the The phenolic compounds were evaluated for their ability to inhibit the growth against *C.albicans* and bacteria *E. coli* and *S. aureus* by the disc diffusion inhibition test contrast to tetracycline which is regarded as standard antibiotic as preliminary test .

The results were explained in table (3) demonstrating how varied phenolic component doses (0.5, 1, and 2,3 mg/ml) improved the inhibitory zone against fungus and bacteria.

The inhibition zone of phenol was 17.5 against *C.albicans* , 13.76 mm against *E .coli*, 12.87 mm against *S. aureus* in concentration 0.5 mg/ml versus 1 mg/ml in concentration in *C.albicans* 18.34 , *S. aureus* 13.98mm , *E.coli* 14.98mm , while concentration 2mg/ml in *C. albicans* 19.21mm , *S. aureus* 14.98mm , *E.coli* 16.87 mm , concentration 3 mg/ml in *C.albicans* 21.65mm *S. aureus* 16.87 mm and *E.coli* 17.98 mm.

The results were explained in table (4) illustrating the impact of various Znonanoparticle concentrations (0.2, 0.5, 0.8, and 1 mg/ml) which increased the inhibition zone against fungi and bacteria .

The inhibition zone of Znonanoparticles was 18.98 against *C.albicans* , 19.76 mm against *E .coli*, 20.76 mm against *S. aureus* in concentration 0.2 mg/ml, while concentration 0.5 mg/ml in *C.albicans* 19.32 , *S. aureus* 21.87mm , *E.coli* 20.54mm , while concentration 0.8mg /ml in *C.albicans* 21.87mm , *S. aureus* 22.87 , *E.coli* 16.87 mm , concentration 1 mg/ml in *C.albicans* 22.98 mm , *S. aureus* 24.8 mm and *E.coli* 23.98 mm.

3. Discussion

The synthesis and accumulation of the natural plant products known as phytochemical in plants have been examined by [26] It is hypothesized that the extract's active components, including flavonoids, alkal - oids, and tannins, are what give it its antibacterial activity. The results of the study [27] which suggested that the active components in plant extracts are highly chemicals that prevent fungi from growing [28].

According to a study, the potently poisonous chemicals that make up plant extracts' active components prevent fungi from growing. Cellular mortality will result from non-specialized interactions between active chemicals in extracts and succinate dehydrogenase and NADH because they will impede the enzymes and cofactors necessary for vital metabolic processes. The active components of the extract, including flavones, alkaloids, and tannins, are thought to be the cause of its antibacterial capability. The results of [29].

Discuss the antibacterial properties of the phenolic component extraction and the therapeutic value of the M. sativa plant. Some molecules involved in secondary metabolism have phenolic rings with a single substitution and the highest possible degree of oxidation. The medicinal herbs include phenols, a powerful antibacterial compound [30]. The oxidized molecule may block enzymes, there may be a reaction with sulfhydryl groups, or there may be more general interactions with proteins that cause phenolic toxicity to microorganisms. [31] Carboxylic acids, which have been shown to be a potent antibacterial agent, were discovered to be associated to numerous antimicrobial and antifungal actions. These acids are known to exist in diverse plant metabolite molecular structures [32]. These result agree with [33], the phenolic extracts at concentration 500 mg/ml gave highest inhibition zone for leaves 25mm, fruits 19mm and barks 21mm against *S. aureus.*

The results showed the ability of ZnO nanop - articles to affect on *S.aureas* and *E.coli* when used in different concentrations and showed the ability of high concentration as compared to little concentration. ability of this Nano material to interact with organic compound of surface wall bacteria and destroy it. That led to destroy the cell wall and death of bacteria [34]. ZnO NPs antibacterial movement specifically relates with their focus as announced by a few examinations, in a similar manner, the action is estimate subordinate. In any case, this reliance is additionally impacted by convergence of NPs. Bigger surface region and higher fixation are responsible for ZnO NPs antibacterial action [35].

Conclusions

Phenol compound and Zno nanoparticles has height inhibition for Antimicrobial.

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